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FOOD STANDARDS AGENCY

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REVIEW OF PAST AND CURRENT RESEARCH ON VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* (VTEC) IN RELATION TO PUBLIC HEALTH PROTECTION

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Title: Review of past and current research on Verocytotoxin-producing *Escherichia coli* (VTEC) in relation to public health protection

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This Review is dedicated to Henry Smith who was a leading member of the project team and whose expertise and wealth of knowledge in this area of research was so valuable in the development and progress of this important review.

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EXECUTIVE SUMMARY

Escherichia coli is a common bacterium found in the intestines of many animals including man. Most strains of *E. coli* are harmless although some strains are capable of causing diarrhoeal disease in humans and some can be responsible for more severe infections and clinical complications. One group of pathogenic *E. coli* is the Verocytotoxin-producing *Escherichia coli* (VTEC), which comprises a large group of *E. coli* serotypes capable of producing Vero cytotoxins (VT), of which there are 2 main types, VT1 and VT2, but also a number of VT2 variants. These toxins are encoded by *vtx* genes carried by bacteriophages which facilitate their transfer between bacteria and also regulate *vtx* expression and toxin production. The most notable VTEC serotype is O157:H7, which was first recognised as a foodborne pathogen in 1982 following hamburger associated outbreaks involving cases of bloody diarrhoea in the USA. Since then over 400 serotypes of VTEC have been recognised, although the clinical significance of many remains unknown. In humans VTEC are responsible for a wide range of clinical manifestations. These include asymptomatic carriage, uncomplicated or mild diarrhoea, haemorrahagic colitis (bloody diarrhoea) and severe complications including haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic pupura (TTP). One feature of HUS is the damage caused to the kidneys which can result in renal failure. The predominant serotype associated with HUS and severe human disease is VTEC O157:H7, although more than 150 non-O157 serotypes have been associated with human disease. These bacteria have emerged to become the most extensively studied group of bacteria of recent times. Our knowledge of these bacteria, their physiology, underlying genetics, the regulation and structure of the phage borne *vtx* genes and the carriage and regulation of virulence associated genes has advanced at an impressive rate. The lack of harmonised research has resulted in similar information being generated which cannot be directly compared. Failure to standardise methods and surveillance systems has also prevented comparison of information from different studies and countries. This review provides a comprehensive account of VTEC research worldwide. It covers every aspect of VTEC research from studies with these bacteria, the characteristics of VTEC, including known virulence associated genes, methodology, clinical aspects of VTEC infection, epidemiology in humans, reservoirs for VTEC in the food chain and prevention and control of VTEC contamination. Through an international network of VTEC researchers and opinion leaders, this review has identified gaps in knowledge and aspects of VTEC research where there is currently insufficient information or a lack of understanding of these bacteria. This review gives a recent account of the published research and the major discoveries relating to VTEC.

ABBREVIATIONS USED IN THIS REVIEW

AA	aggregative adherence
AAF	aggregative adherence fimbriae
A/E	attaching and effacing
ACMSF	Advisory Committee on the Microbiological Safety of Food
AEEC	attaching effacing *Escherichia coli*
AFSSA	Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency)
AMI	American Meat Institute
AMIF	American Meat Institute Foundation
AOAC	Association of Official Analytical Chemists
ATP	adenosine triphoshate
AU	active units
BAM	Bacteriological Analytical Manual
BBSRC	Biotechnology and Biological Sciences Research Council
BFP	bundle forming pili
bp	base pair
CCFRA	Campden & Chorleywood Food Research Asssociation
CDT	cytolethal distending toxin
CDC	Centers for Disease Control and Prevention
cfu	colony forming units
CFs	colonisation factors
CFAs	colonisation factor antigens
Da	dalton
DALYs	disability adjusted life years
DAEC	diffusely adherent *Escherichia coli*
DH	Department of Health
Defra	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
eae	*Escherichia coli* attaching and effacing gene
EAF	EPEC adherence factor
EAST	enteroaggregative heat-stable toxin
EHEC	enterohaemorrhagic *Escherichia coli*
ExPEC	extraintestinal pathogenic *Escherichia coli*
EPEC	enteropathogenic *Escherichia coli*
EIEC	enteroinvasive *Escherichia coli*
ETEC	enterotoxigenic *Escherichia coli*
EAggEC	enteroaggregative *Escherichia coli*
ECDC	European Centre for Disease Prevention and Control
ELISA	enzyme linked immunosorbent assay
Efa	EHEC factor for adherence
EFSA	European Food Safety Authority
Esp	*E. coli* secreted protein
ESRD	end-stage renal disease
FSA	Food Standards Agency
FDA	United States Food and Drug Administration
FSIS	Food Safety Inspection Service
Gb3	globotriaosylceramide (receptor)
Gb4	globotetraosylceramide (receptor)

GUD	β-glucuronidase
HACCP	Hazard Analysis Critical Control Points
HPA	Health Protection Agency
HC	haemorrhagic colitis
HUS	haemolytic uraemic syndrome
HUVEC	human umbilical vein endothelial cells
IPEC	intestinal pathogenic *Escherichia coli*
IPRAVE	International Partnership Research Award in Epidemiology
ISO	International Organisation for Standardisation
kb	kilobase
kDa	kilodalton
LPS	lipopolysaccharide
LEE	locus of enterocyte effacement
ler	LEE encoded regulator
LT	heat labile enterotoxin
MAP	mitogen activated protein (kinase)
MIC	minimum inhibitory concentration
M	molar
mL	millilitre
MLEE	multilocus enzyme electrophoresis
mM	millimolar
MRC	Medical Research Council
μM	micromolar
MW	molecular weight
MPN	most probable number
MSFFG	Microbiological Safety of Food Funders Group
NSF	non-sorbitol fermenting
ng	nanogram
nt	nucleotide
OBGS	octames-based genome scanning
OCA	Ontario Cattlemans Association
OD	optical density
OMP	outer membrane protein
PAI	pathogenicity island
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pg	picogram
Per	plasmid encoded regulator
Pet	plasmid-encoded toxin
pfu	plaque forming units
PMN	polymorphonuclear leukocyte
Pss	protease secreted by STEC
PFGE	pulsed field gel electrophoresis
REPEC	rabbit specific enteropathogenic *E. coli*
RFLP	restriction fragment length polymorphism
RPLA	reverse passive latex agglutination
RNA	ribonucleic acid
ROI	Republic of Ireland
SAC	Scottish Agricultural College
SCIEH	Scottish Centre for Infection and Environmental Health

SEERAD	Scottish Executive for the Environment and Rural Affairs Department
Saa	STEC autoagglutinating adhesin gene
SF	sorbitol fermenting
ShET	*Shigella* enterotoxin
SPATE	serine protease autotransporters of the Enterobacteriaceae
ST	heat-stable enterotoxin
STEC	Shiga-toxin *Escherichia coli*
Stx	Shiga toxin
stx	Shiga toxin gene
TccP	Tir-cytoskeleton coupling protein
Tir	translocated intimin receptor
TTP	thrombocytopoenic purpura
TTSS	type III protein secretion system
TNS	tumour necrosis factor
USDA	United States Department of Agriculture
VBNC	viable but non-culturable
VCA	vero cell assay
VLA	Veterinary Laboratories Agency
VT	Verocytotoxin
vtx	Verocytotoxin gene
WHO	World Health Organisation
μg	microgram